TOWARDS A GOAL OF AUTOMATED GLYCOPROTEOMIC ANALYSIS

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University of New Hampshire

Follow-on Biologics Workshop; 12- 12, 2005

Follow-On Strategies for Carbohydrate Sequencing

1. **Mining Structural Details by MS*n*.**
   Dave Ashline, et al., Anal. Chem. 77:6250 (2005)

2. **FragLib: An MS*n* Spectral Library.**
   Hailong Zhang, H. et al., Anal. Chem. 77:6263 (2005)

3. **An Algorithm for Assigning Topology from MS^n Data.**
   Tony Lapadula, et al., Anal. Chem. *77*:6271 (2005)

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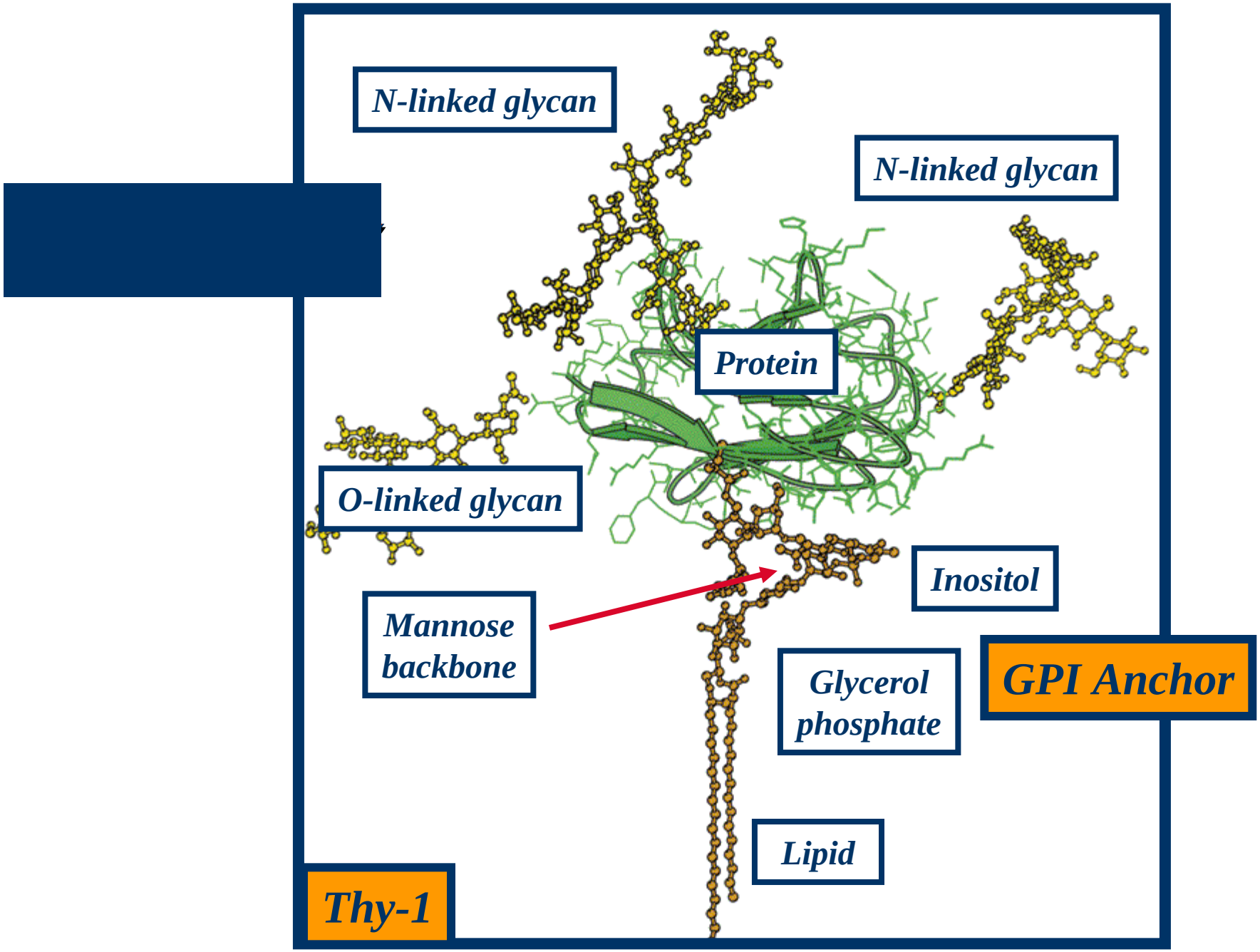
http://glycomics.unh.edu

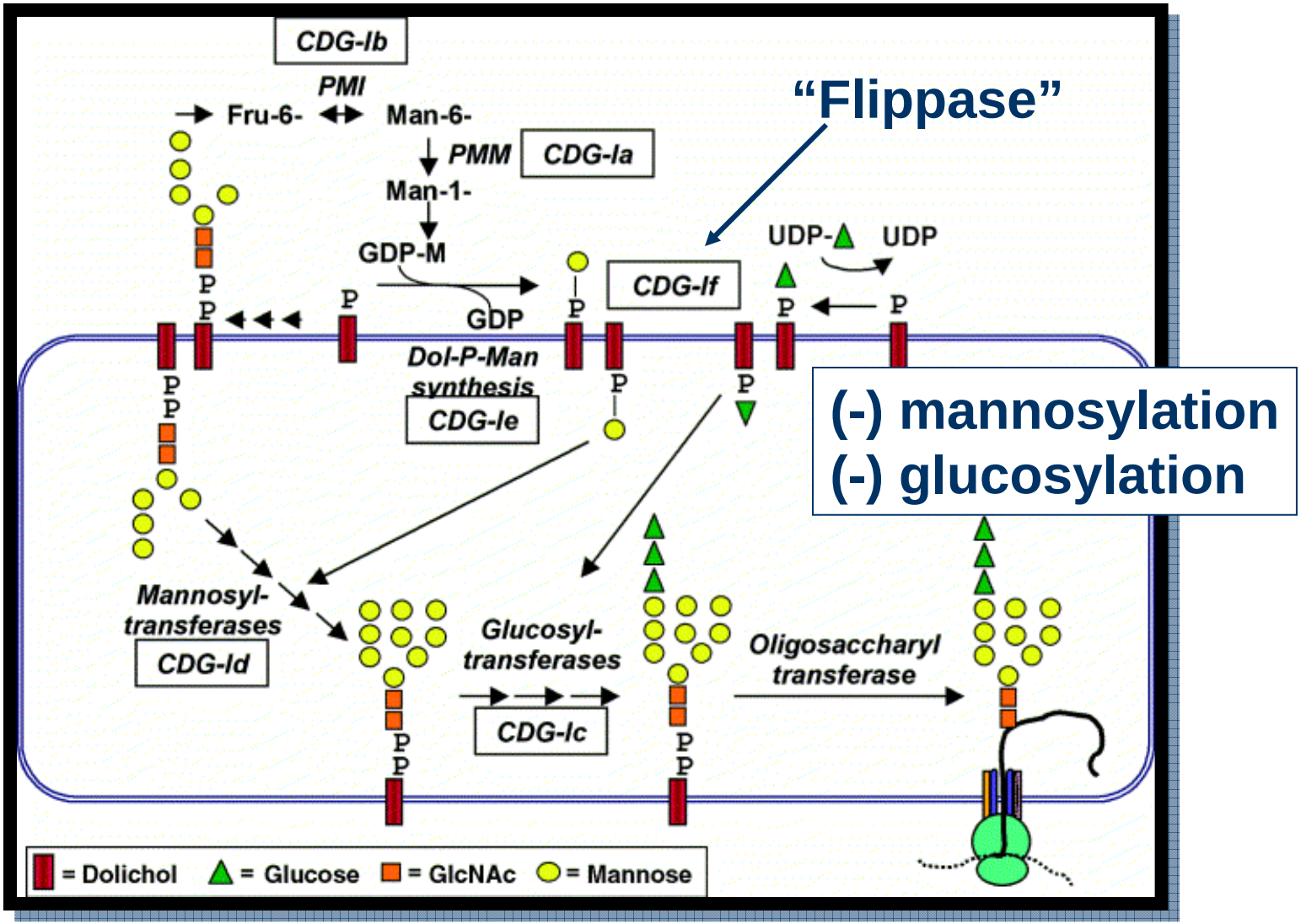
NIGMS and NCRR

Emil Fischer, Nobel Lecture 1902

…….study also dates back to the early beginnings of organic chemistry …. Nevertheless more than a century elapsed from the elucidation of their elementary composition by Lavoisier before science prepared them by artificial means. *The reason for this slow progress lies firstly in the peculiar difficulties which those substances pose for experimental treatment, and secondly in their great profusion of forms which also necessitates a rather complex systematology.*


N-linked glycan
N-linked glycan
Protein
O-linked glycan
Inositol
Mannose backbone
Glycerol phosphate
GPI Anchor
Lipid
Thy-1

Inborn Glycosylation Errors



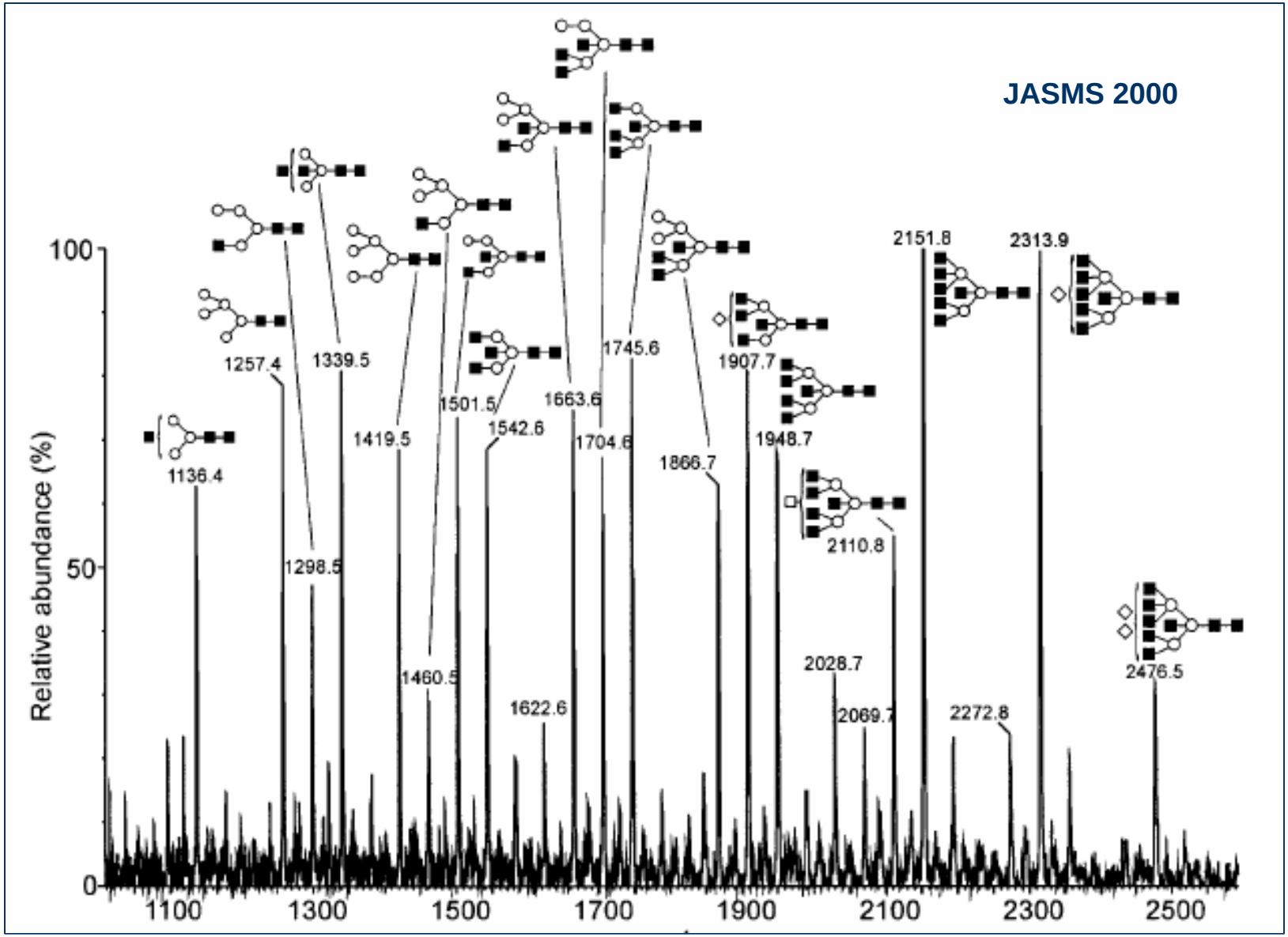
Clinical phenotype of the CDG-If patient at 11 years of age. The child is unable to stand or walk without support.

Clinical features of CDG patients Part I

Neurology	Axial hypotonia; hyporeflexia; developmental delay; seizures; stroke-like events
Gastroenterology/ hepatology	Failure to thrive; diarrhoea; protein-losing enteropathy; liver dysfunction; vomiting; hepatomegaly; cholangitis
Neonatology	Ascites; hydrops; multiorgan failure
Haematology	Thrombocytosis; thrombocytopenia; coagulopathy; thrombosis
Endocrinology	Hyperinsulinemic hypoglycemia; hypothyroidism; hypogonadism
Clinical genetics	Dysmorphic features; microcephaly
Orthopaedics	Osteopenia; joint contractures; kyphosis/scoliosis
Ophthalmology	Abnormal eye movements; squint; cataract; retinitis pigmentosa; nystagmus; iris coloboma; cortical blindness

De Lonlay, et al, 2001

Glycan Profile Ovalbumin; Hz Released MALDI-MS

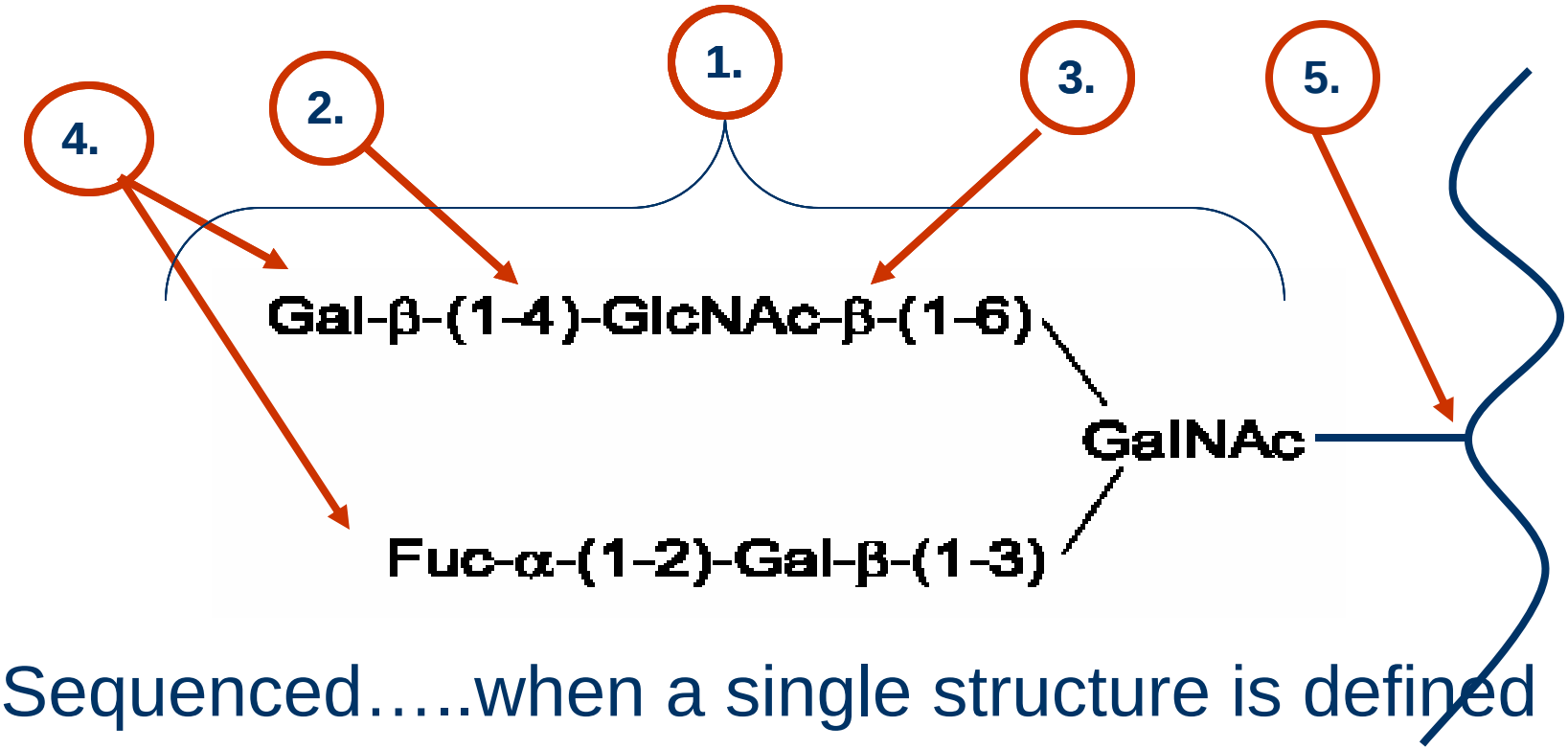


Component Strategies for Molecular Detail

1. **Effective Release and Profiling**
2. **Methylation; Structural Detail Upon CID**
3. **Metal Adduction; Charge & Stereochemistry**
4. **Multi-dimensional Analysis, MS^n**
5. **Data Handling; Library Comparison & Filing**
6. **Reassembly of Pieces; Algorithm**

Goals for a Full Understanding of Structure

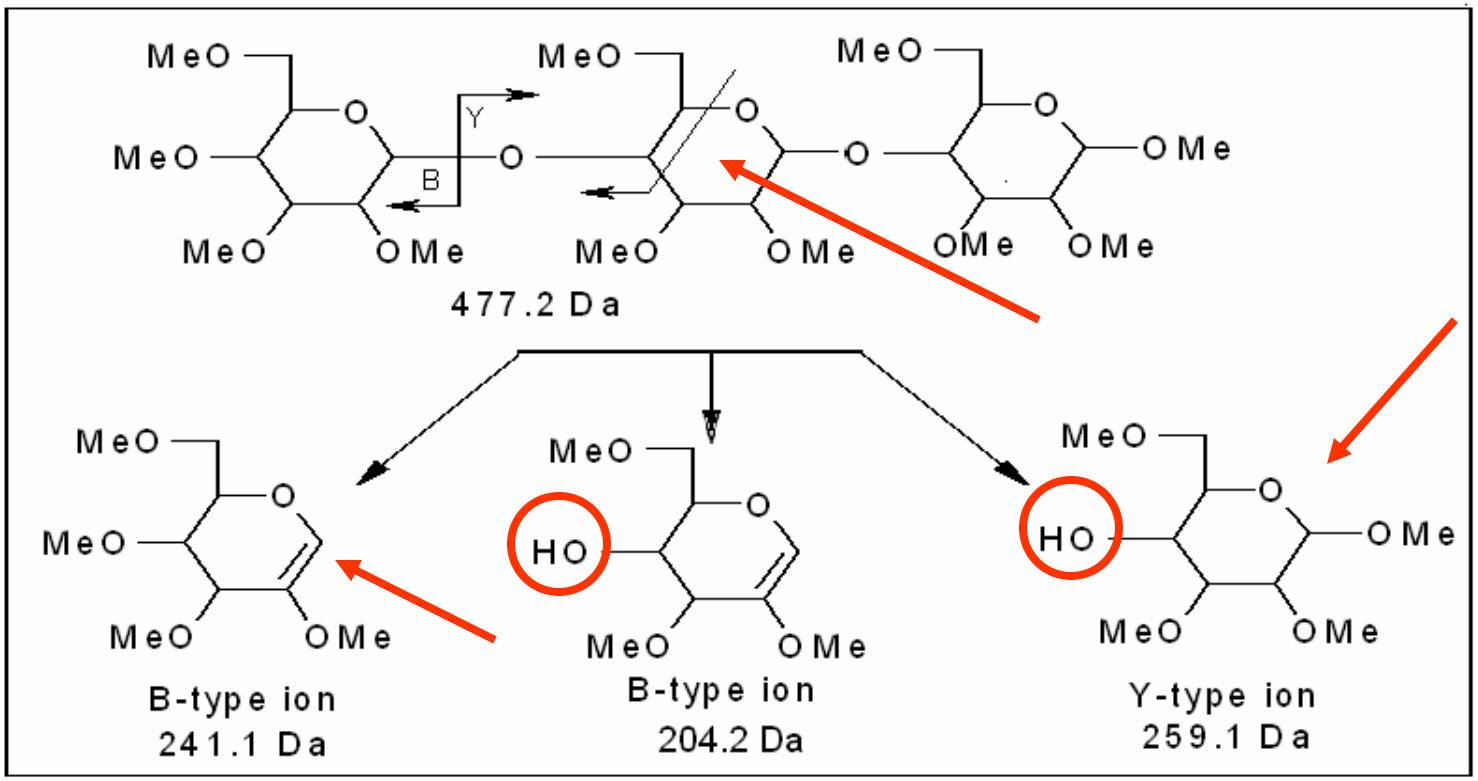
1. Oligomer Topology, linear, branching, (isobars ID)
2. Inter-residueLinkage
3. Anomer (αor β) Stereochemistry
4. Monomer Identification
5. N-, O-Linked Location, (site occupancy)



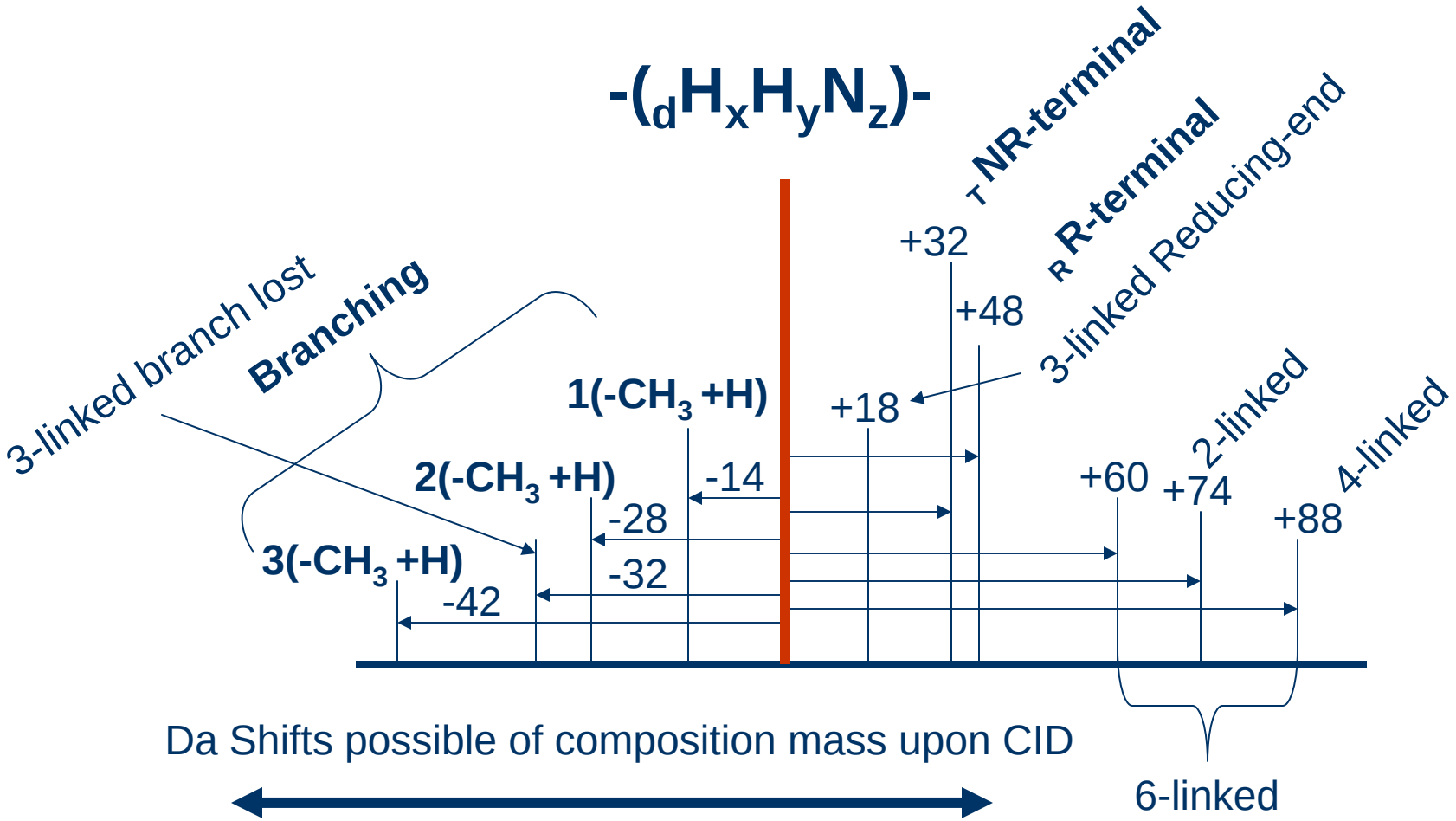
Sequenced…..when a single structure is defined

Methylation and Topology

Stereospecificity and Connectivity



Fragment Mass Composition (*in silico* composition libraries)



Strategies Toward Automated Glycan Sequencing
--- Unified Methodoloogy ---

“Top Down”
(bioinformatics)
“OSCAR algorithm”

-Branching
-Some linkage
-Isobars

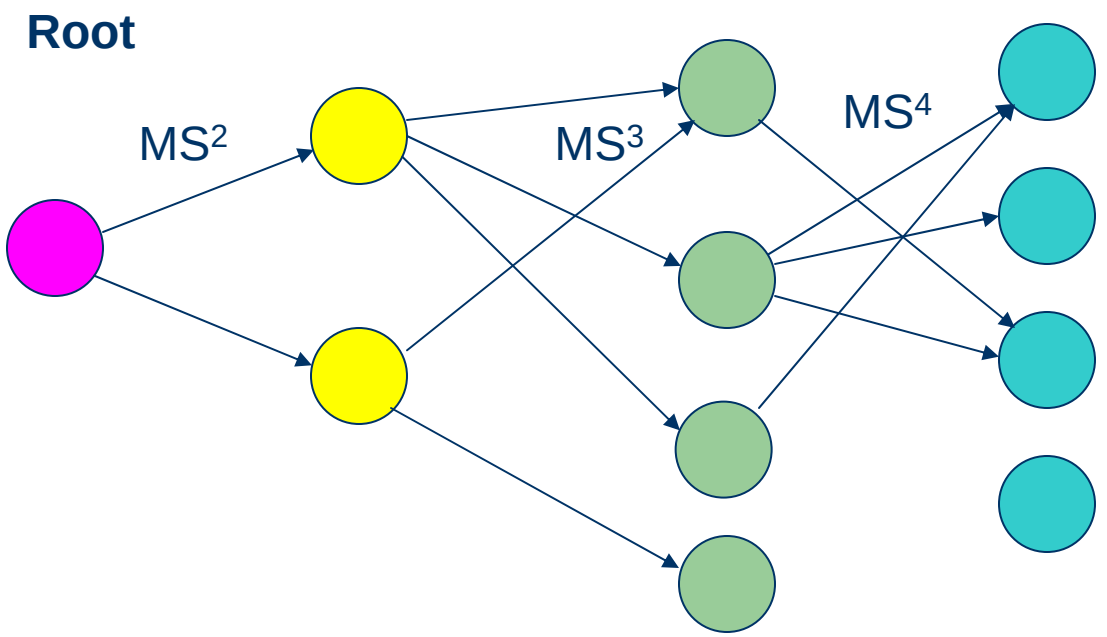
Total Structure by MS^n

-Linkage
-Monomers
-Anomericity
-Isobars

“Bottom up”
(Mining MS^n)
“FragLib”

http://glycome.unh.edu/

MS^n data tree:



MS^n data structure (tree) reflects glycan structure

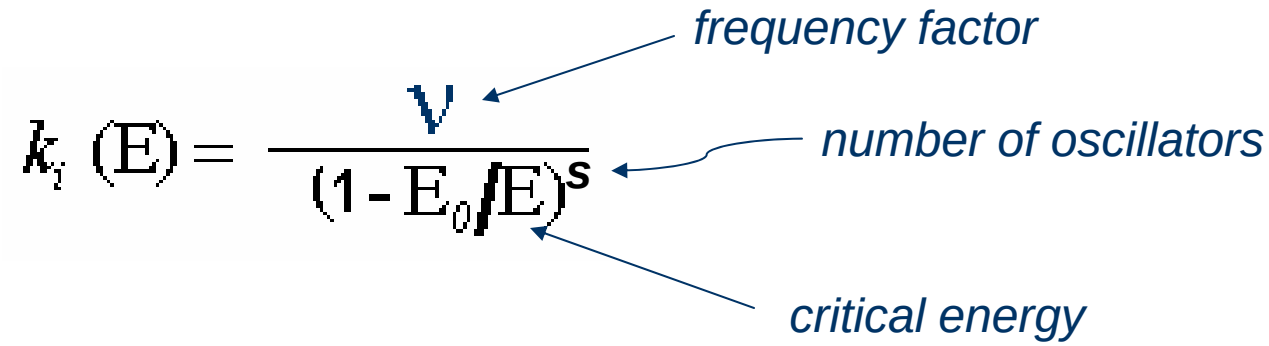
Theory of mass spectra

1. Quasi-equilibrium theory (QET)

1) Molecular ions in various electronic (and vibrational) states are produced by EI (or PI).

2) Ions in excited electronic states undergo rapid internal conversion to the ground state. → Rapid conversion of electronic energy to vibrational energy.

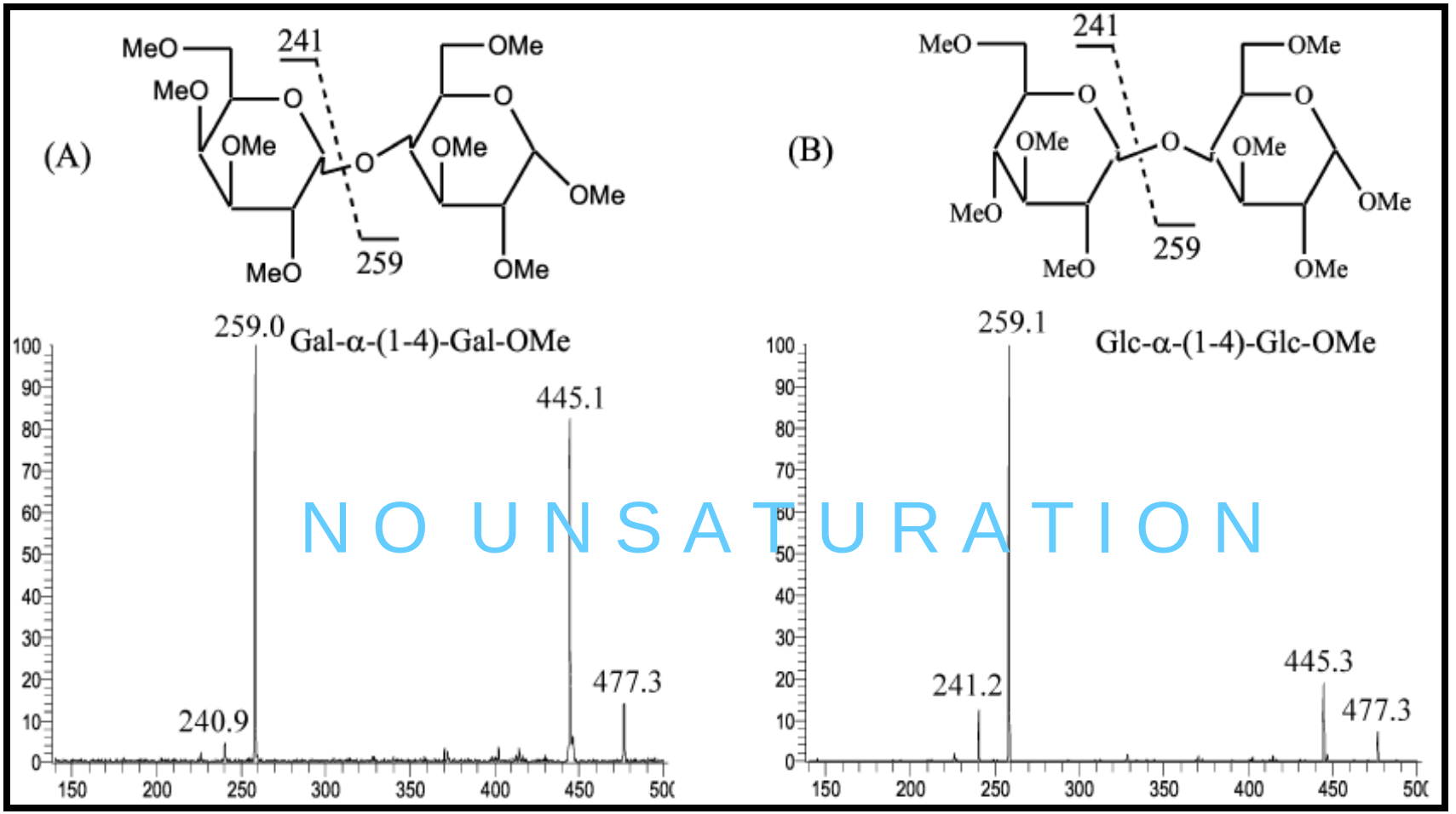
3) Intramolecular vibrational redistribution (IVR) occurs rapidly also. → Transition state theory, or, Rice-Ramsperger-Kassel-Marcus (RRKM) theory. ⇒ QET or RRKM – QET



$$k_i(E) = \frac{\nu}{(1 - E_0/E)^s}$$

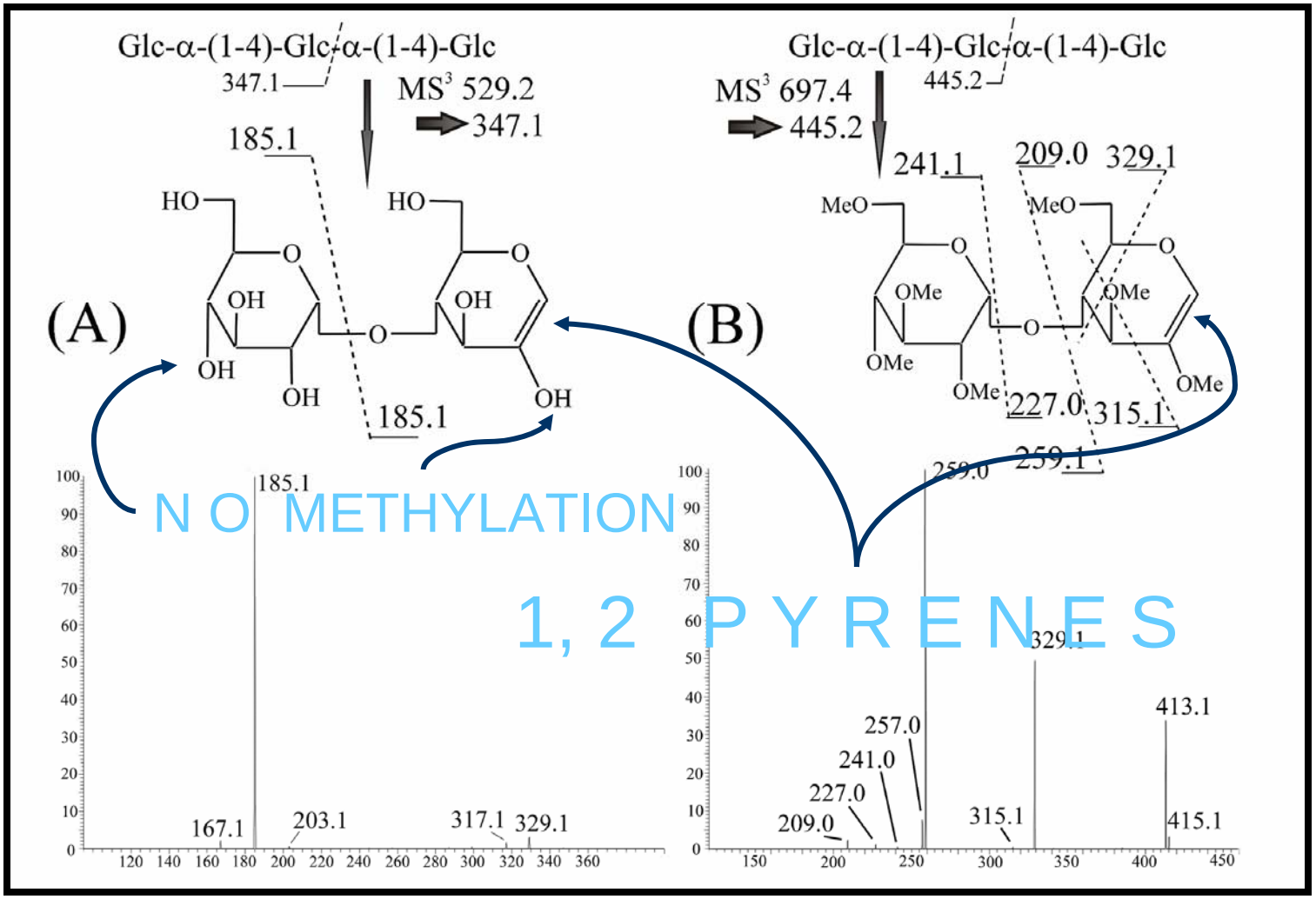
MS^2 Identity with Methylglycoside Isobars

(gal-4gal vs glc-4glc)

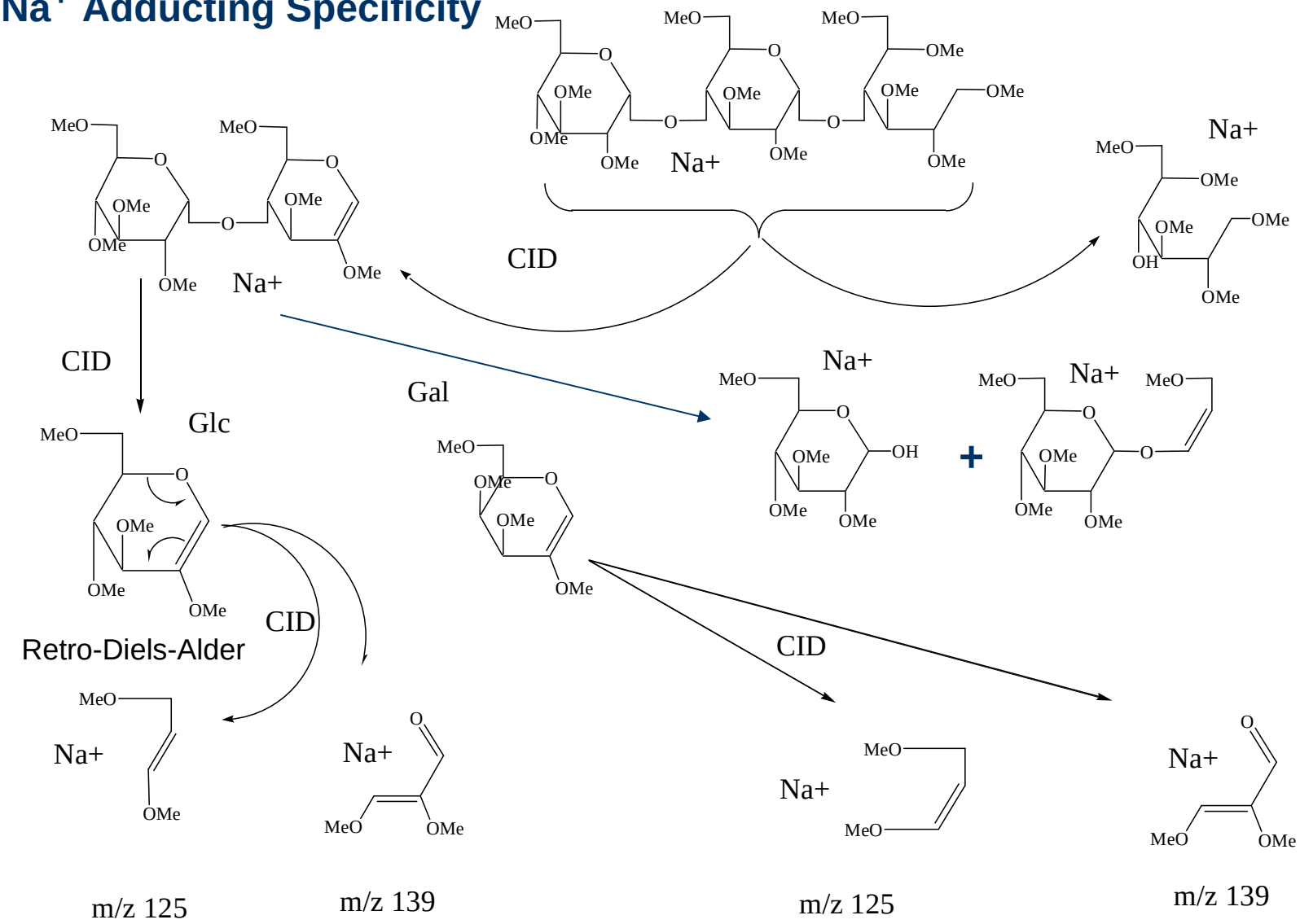


Methylation and Pyran-1-ene Disaccharides

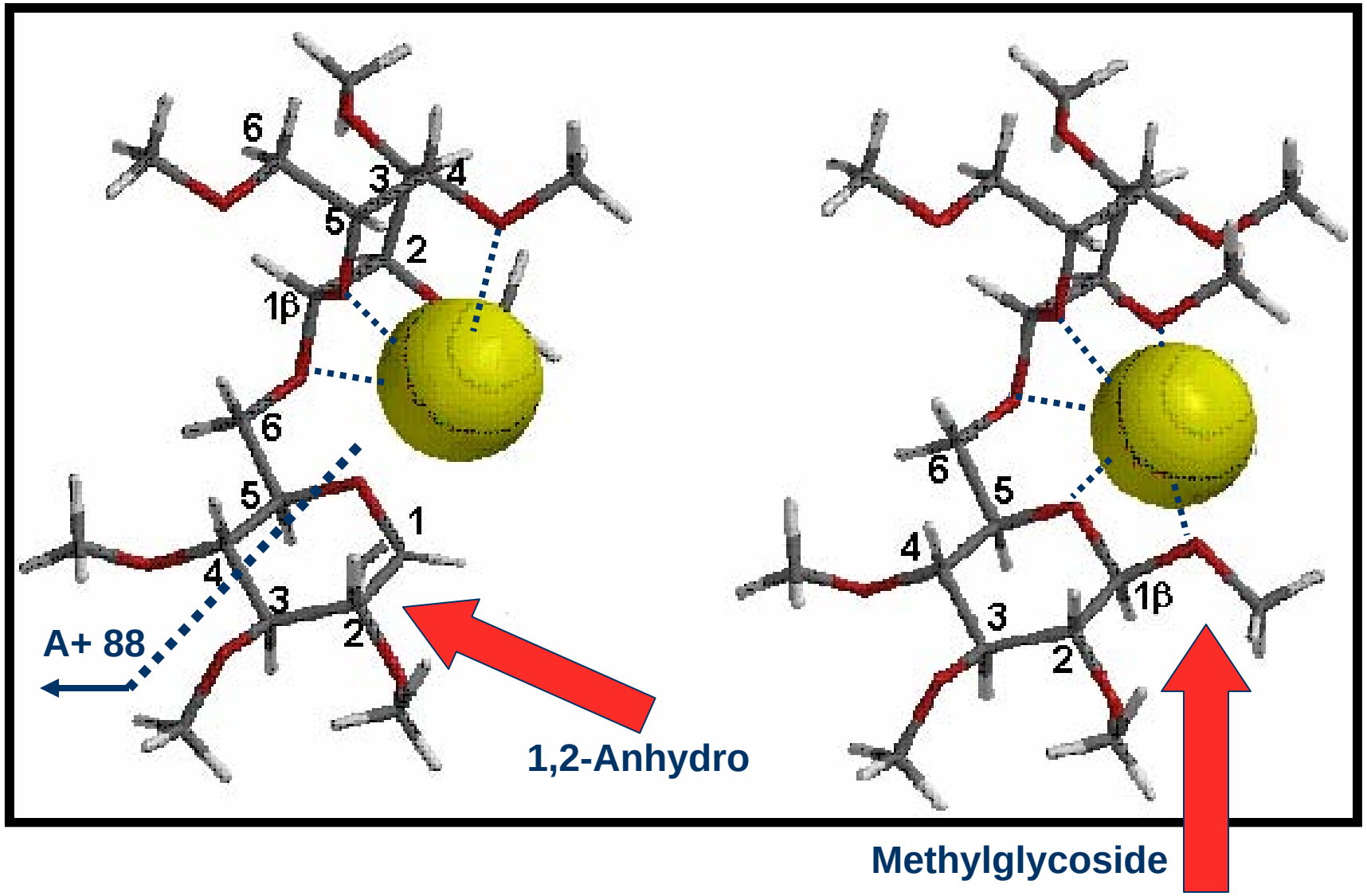
(glc-4glcene vs permethyl-glc-4glcene)



Na^+ Adducting Specificity

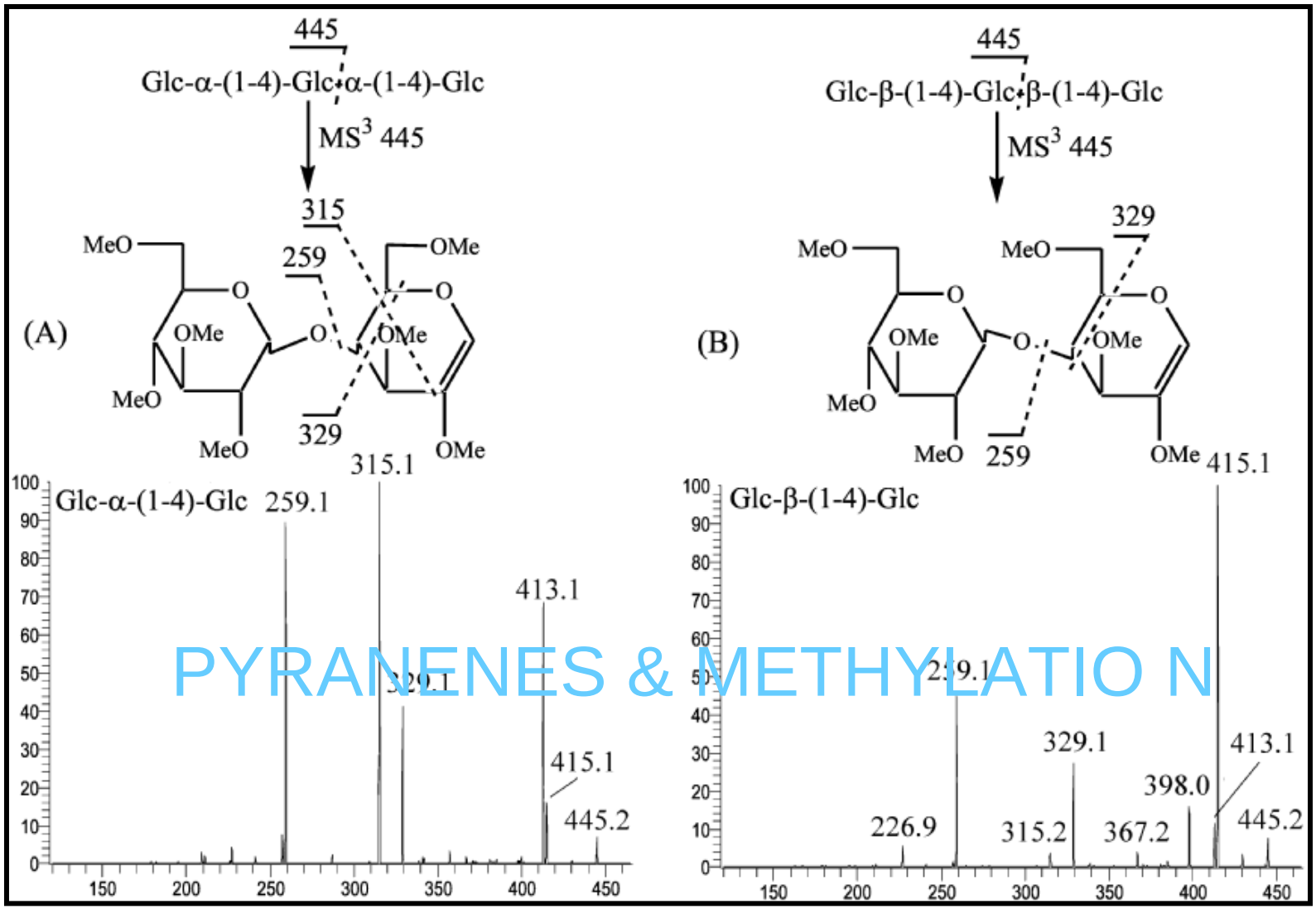


Stereospecific Metal Ion Adduction

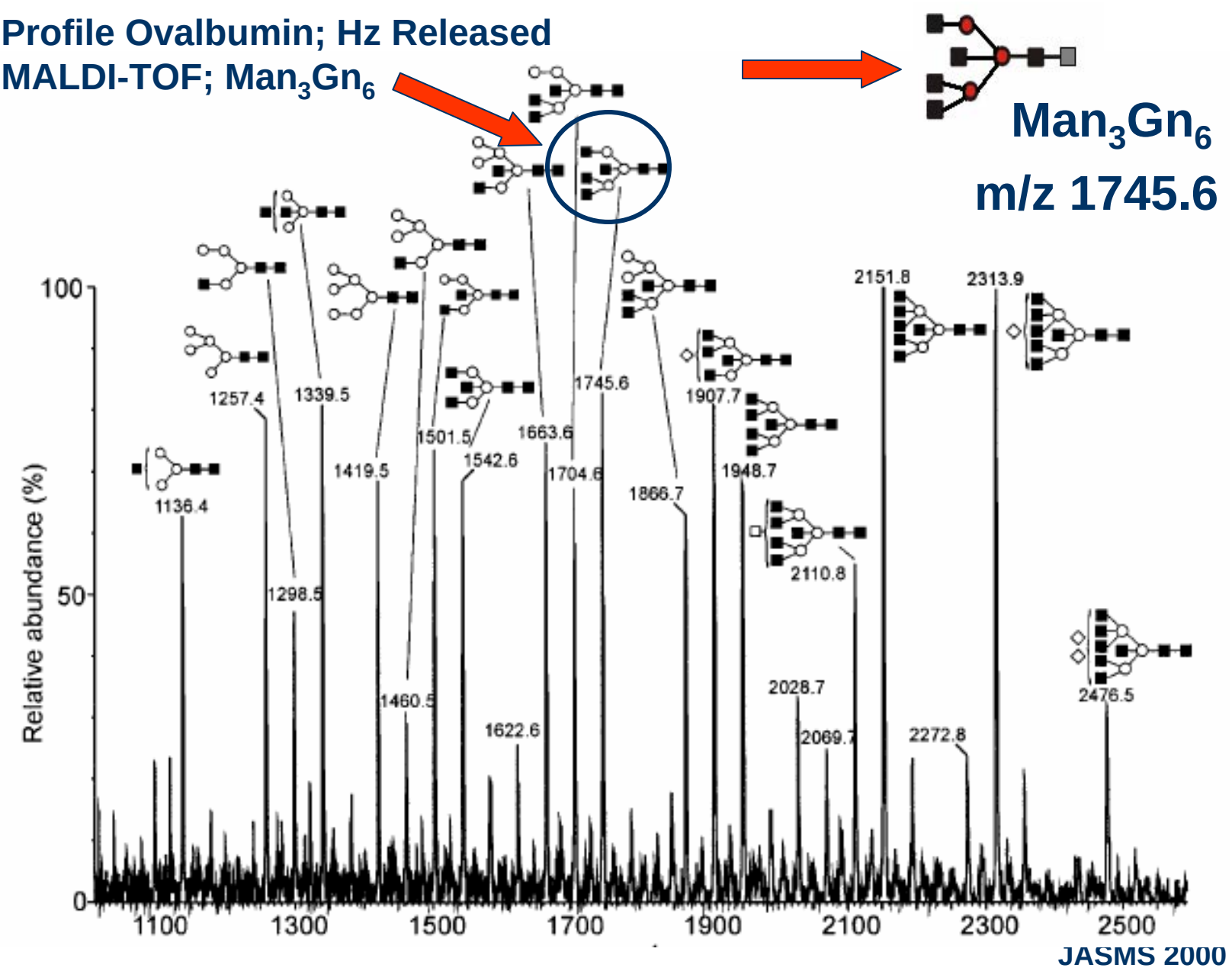


Anomers: MS^3 Non-identity with 1,2-ene

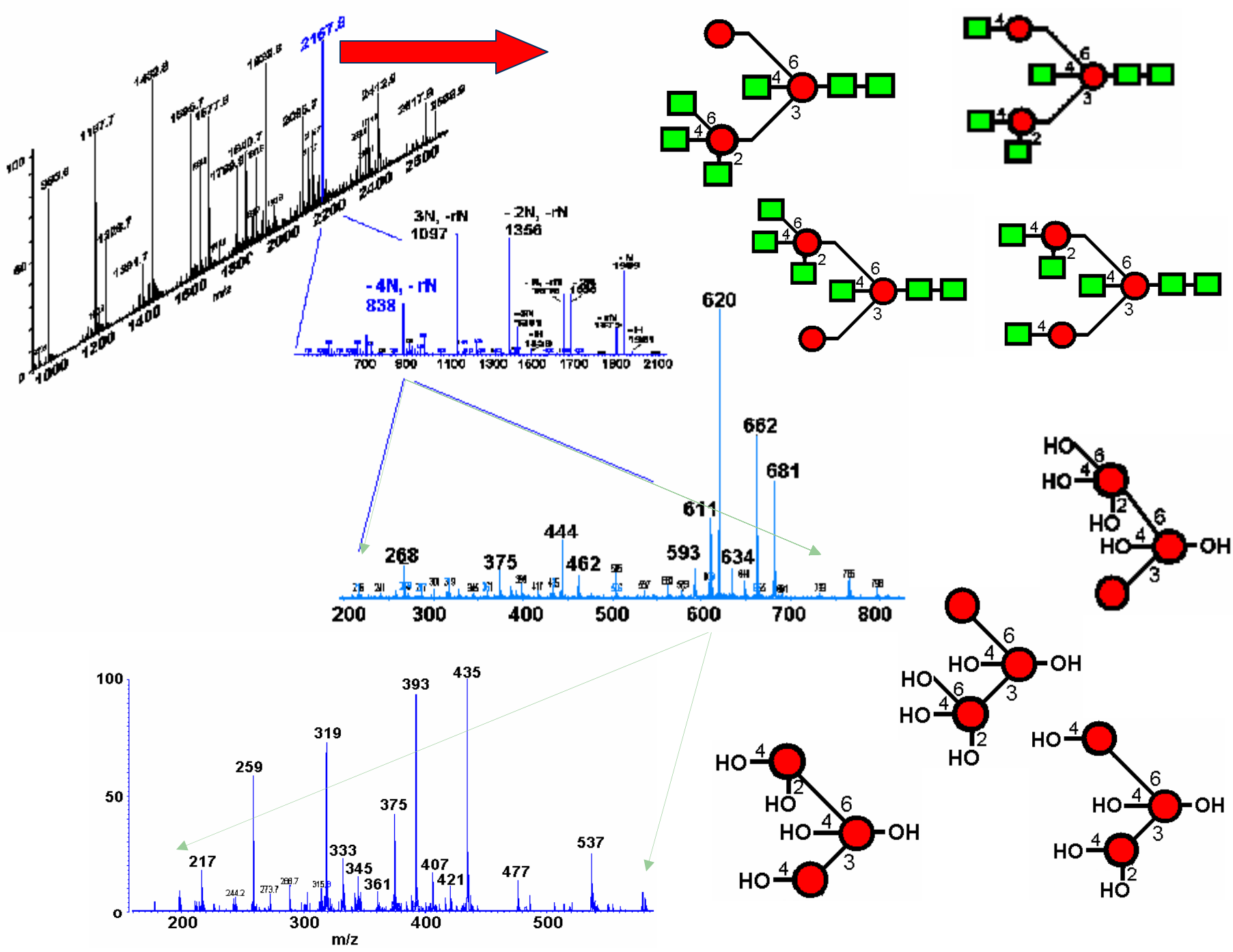
glc-α4glc vs glc-β4glc

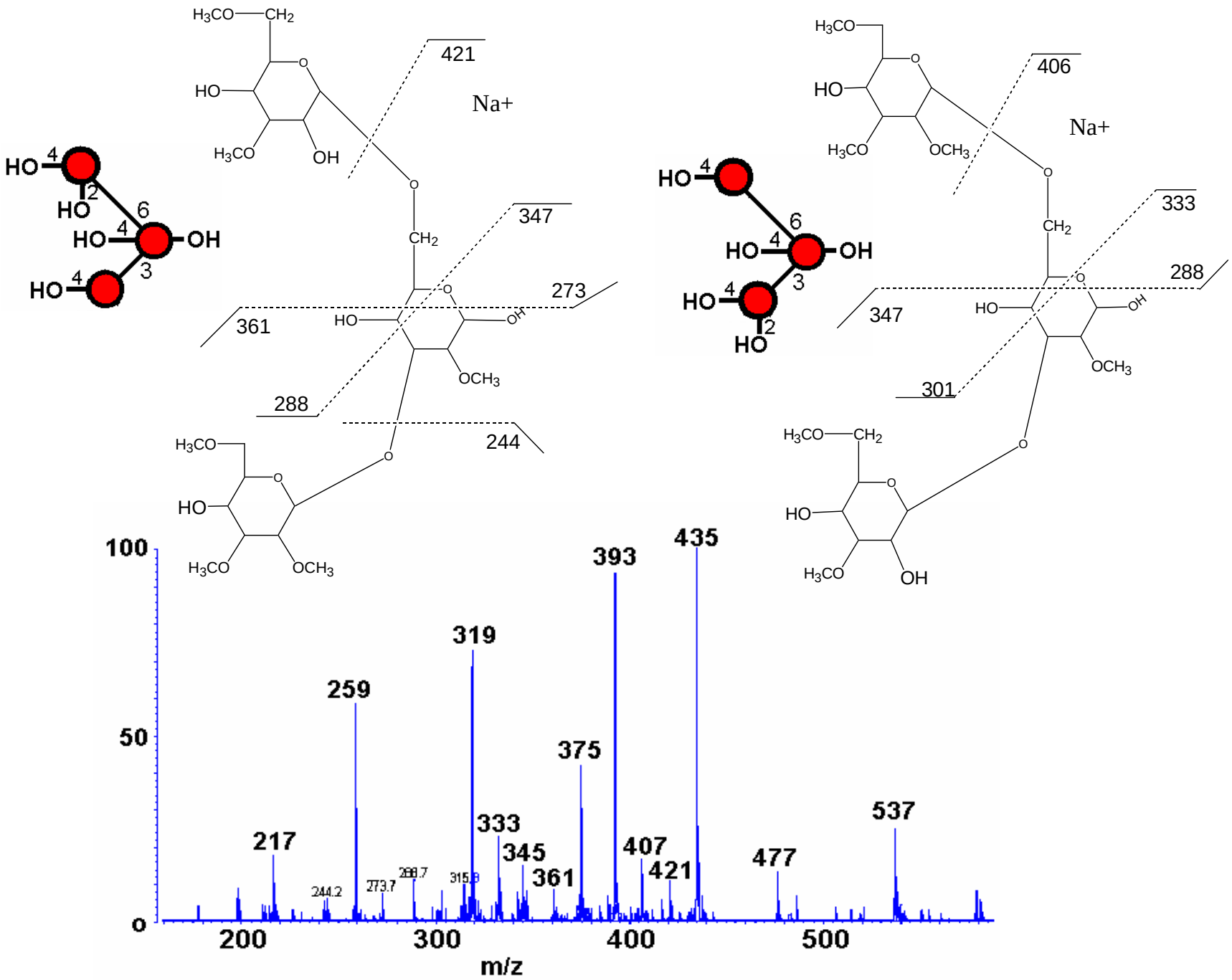


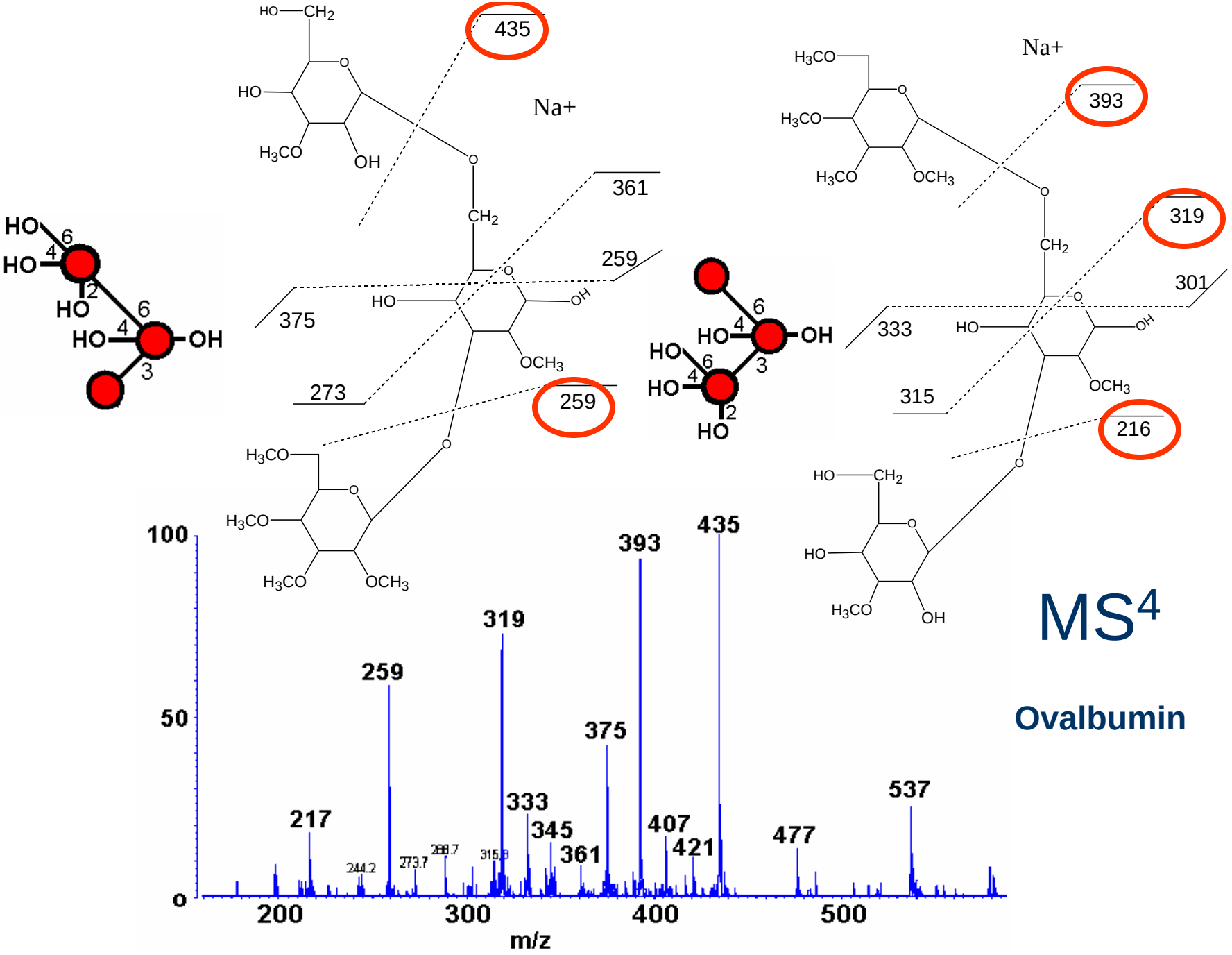
Profile Ovalbumin; Hz Released
MALDI-TOF; Man_3Gn_6



JASMS 2000


2167.8
3N, -rN
1097
-2N, -rN
1356
-4N, -rN
838
620
662
681
611
444
268
375
462
593
634
HO
OH
100
50
0
393
435
319
259
375
333
345
361
407
421
477
537
217
m/z


421
Na+
347
273
361
288
244
406
Na+
333
288
347
301
100
50
0
200
300
400
500
m/z
435
393
319
259
375
537
333
345
407
421
361
477
217
244.2
273.7


435
Na+
361
259
375
273
259
393
Na+
319
301
333
315
216
100
50
0
200
300
400
500
m/z
435
393
319
259
375
333
345
361
217
407
421
477
537
MS4
Ovalbumin

Component Strategies for Molecular Detail

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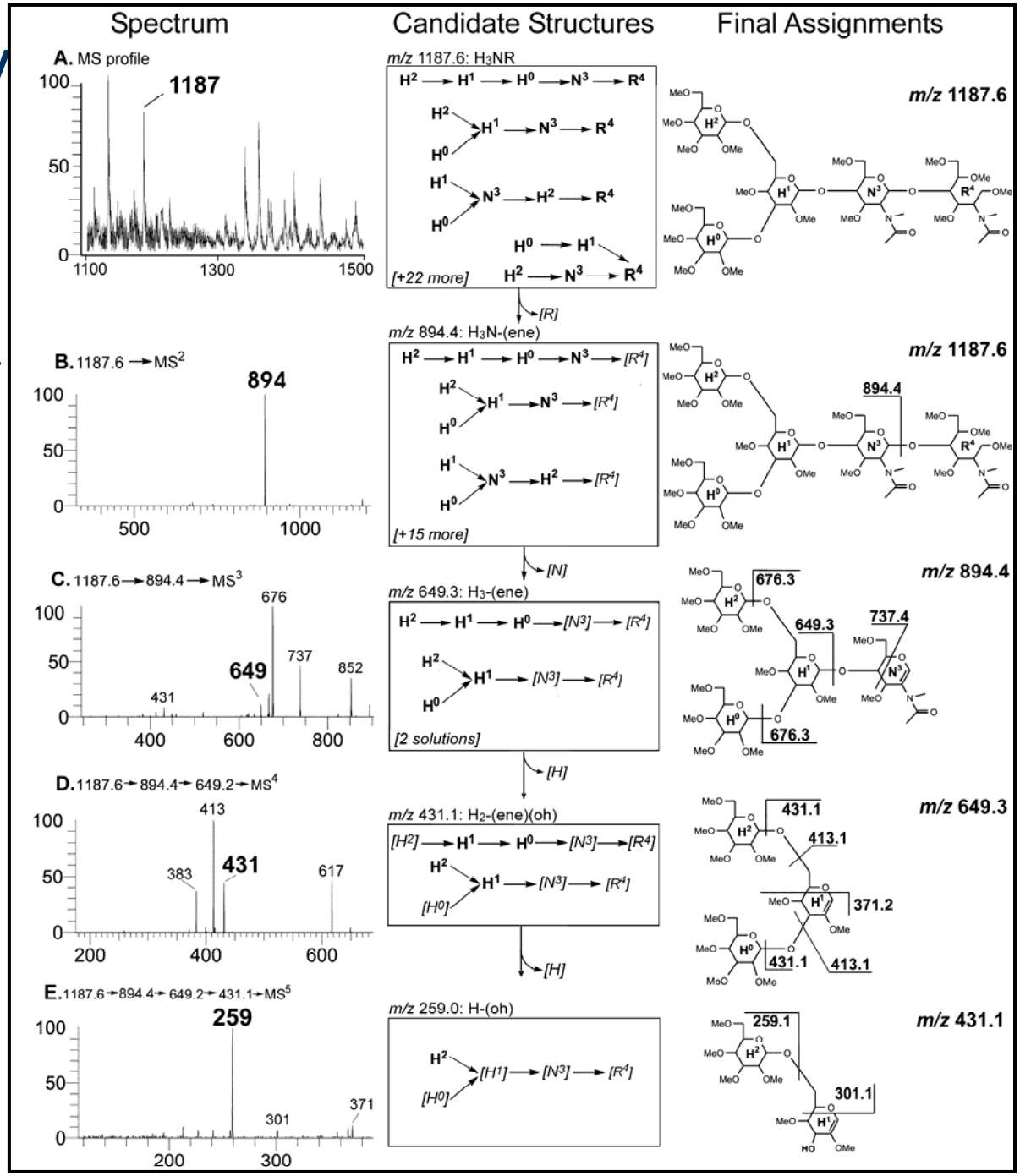
De novo topology assignment by OSCAR

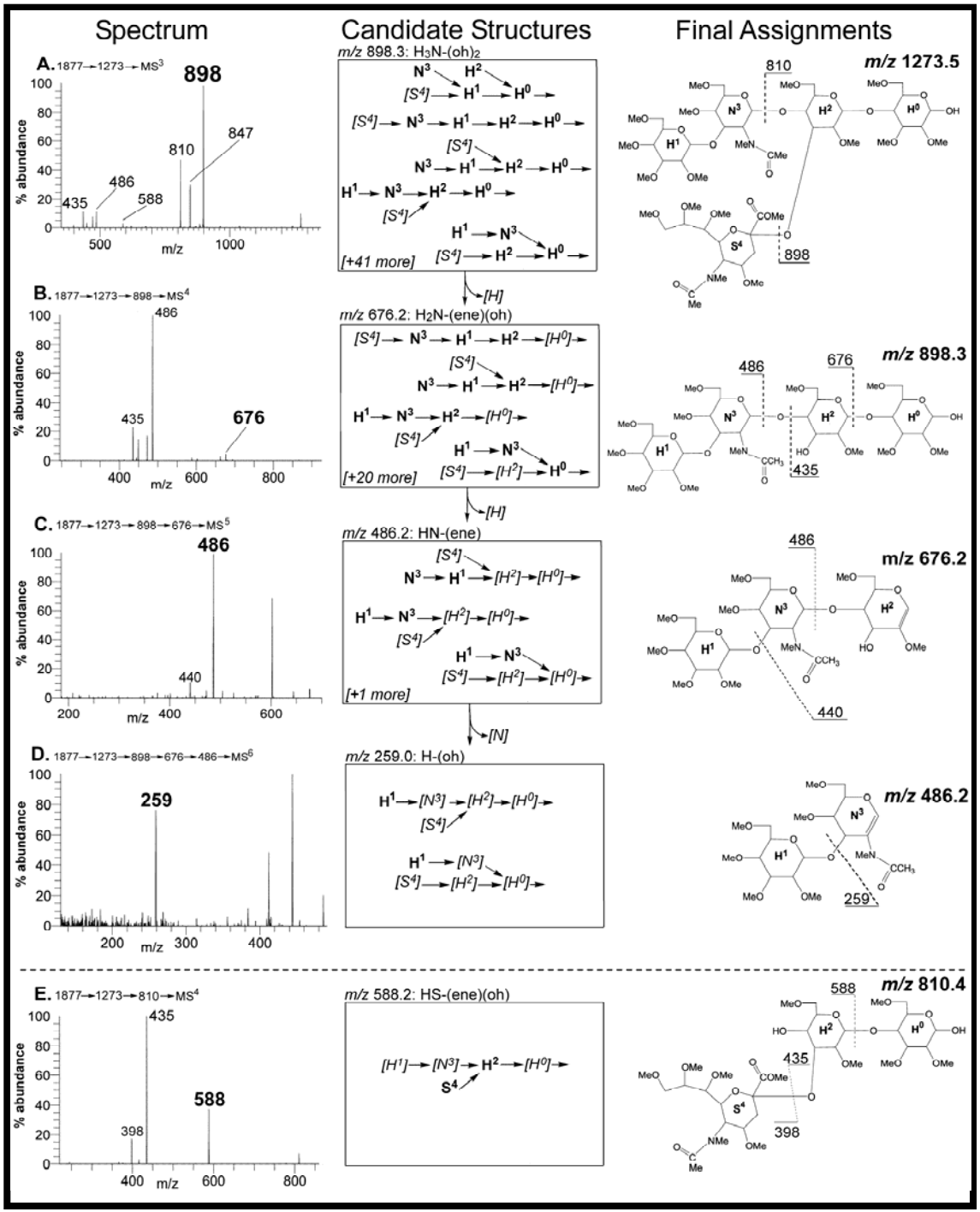
Left column → Spectra
Right column →
Structures
Center column →
Topologies
assigned by OSCAR

MS^1- 26
MS^2- 18
MS^3- 4
MS^4- 2
MS^5- 1



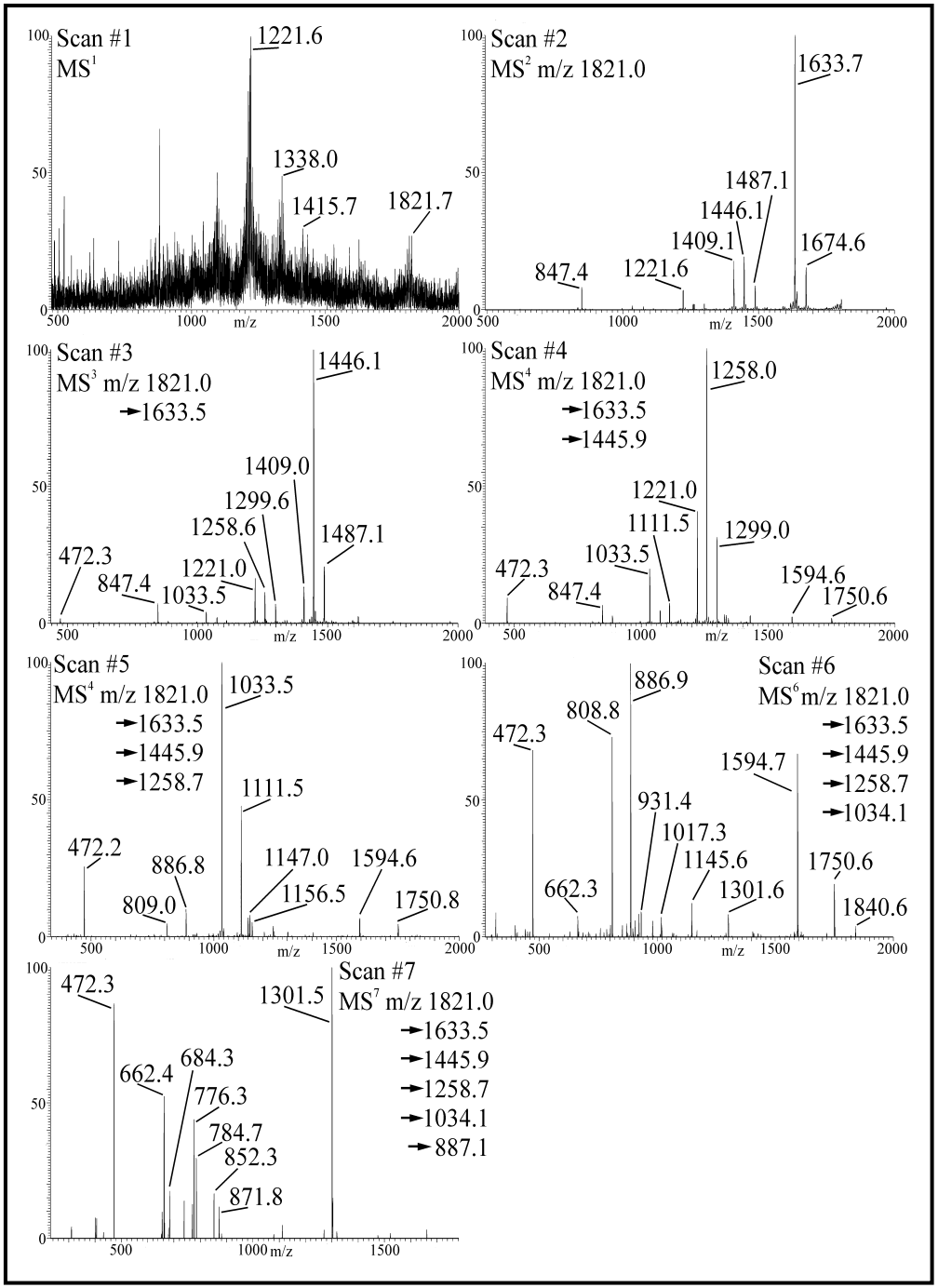
De novo topology assignment of a ganglioside by OSCAR

Topologies assigned
MS^3- 46
MS^4- 24
MS^5- 4
MS^6- 2
Additional MS^4 pathway- 1



Example of Sequential Mass Spectra Collected Automatically on a Reduced, Permethylated Fetuin Glycan

Instrument method was created Using Xcalibur General MS or MSn experiment
All 8 spectra were obtained in 1.2 minutes on LTQ, peaks were selected by neutral losses, each scan is the average of 100 microscans



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Charles Warren Workshop on Glycoconjugate Analysis

(1st Annual)

~~ A dialog among the specialists ~~

On the Campus of the University of New Hampshire

Sunday Evening July 9 – Wednesday Noon, July 12, 2006

~dinner at the winery~

***

"Can We Find Synergy in Glycoconjugate Analysis?

****

Discussion Sessions in Selected Topics of Structure

High Throughput Strategies; is there a Possibility?
How do you Deal with Glycoproteomics, Glycolipidomics?

Bioinformatics, Bioinformatics, Bioinformatics

ASMS, HUPU, Glycobiology Soc.

Logistics, organization, further details; contact

http://Glycomics.unh.edu

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